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Nutritional Relationships in Schistosomiasis

Frank M. Fisher, Jr.

for  
Clark P. Read

July, 1978

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Amino acids, sugars, purines and pyrimidines enter the tegument of adult <u>Schistosoma mansoni</u> by both diffusion and mediated processes. In all examples the mediated processes are specific and demonstrate saturation kinetics as well as inhibition by closely related molecular species. The concentration of amino acids in mouse hepatic portal serum approximated the respective transport constants ( $K_t$ ) determined <u>in vitro</u> . Purines and pyrimidines also enter the adult parasite by both mediated and diffusion processes. Five distinct though complex transport loci were identified.			

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20. (cont.) Nucleotides are primarily dephosphorylated at the tegumental surface prior to permeation. The phosphohydrolase is not involved in the hydrolysis of sugar phosphates nor is it (they) sensitive to  $\text{Na}^+$  for cysteine. Like the above organic solute, the sugars enter the parasite by a mixed mode--both diffusion and mediated systems being utilized. The mediated system is apparently  $\text{Na}^+$  dependent and sensitive to presence of ouabain, phlorizin, phloretin. Hexose mediated accumulation is  $\text{Na}^+$  coupled.

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## SUMMARY

Amino acids, sugars, purines and pyrimidines enter the tegument of adult Schistosoma mansoni by both diffusion and mediated processes. In all examples the mediated processes are specific and demonstrates saturation kinetics as well as inhibition by closely related molecular species. The concentration of amino acids in mouse hepatic portal serum approximated the respective transport constants ( $K_t$ ) determined in vitro. Purines and pyrimides also enter the adult parasite by both mediated and diffusion processes. Five distinct though complex transport loci were identified. Nucleotides are primarily dephosphorylated at the tegumental surface prior to permeation. The phosphodhydrolase is not involved in the hydrolosis of sugar phosphates nor is it (they) sensitive to  $\text{Na}^+$  for cysteine. Like the above organic solutes, the sugars enter the parasite by a mixed mode--both diffusion and mediated systems being utilized. The mediated system is apparently  $\text{Na}^+$  dependent and sensitive to presence of ouabain, phlorizin, phloretin. Hexose mediated accumulation is  $\text{Na}^+$  coupled.

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Statement of the problem.

The nutritional mechanisms of Schistosoma mansoni have been investigated with reference to amino acids and sugars. Specifically, the following questions concerning the feeding mechanism of this parasite have been answered:

- (a) Are significant amounts of free amino acids absorbed by S. mansoni at physiological concentrations?
- (b) Does absorption occur by diffusion or by specific mediated processes?
- (c) Does competition between different molecular species of amino acids occur in absorption?
- (d) Does absorption occur through the syncytial tegument?
- (e) How do absorption mechanisms of the parasite differ from those of the host?
- (f) How do free amino acids available to the adult worm in the portal blood vary as a function of the feeding cycle of the host?
- (g) Do schistosomes affect the free amino acid content of portal blood?
- (h) Is the tegument the major organ for sugar absorption?
- (i) How does the stereospecificity of sugar absorption compare with specificity of hexokinases?
- (j) Is glucose absorption the limiting step in carbohydrate metabolism?
- (k) How does sugar transport specificity of the parasite compare with that of the host?
- (l) Does amino acid and sugar transport specificity suggest new chemotherapeutic attacks on the parasitism?

The following hypotheses have been formulated:

- (a) Amino acid transport occurs through the syncytial tegument of Schistosoma and differs in specificity from transport in host tissues. Previous studies in this laboratory with Fasciola hepatica showed that a number of amino acids enter the parasite by simple diffusion (Isseroff and Reed, 1969). We have found that, while alanine enters S. mansoni by diffusion, lysine enters the worm by a specific mediated system. Further, it enters through the tegument. This strongly supports the general hypothesis stated above. A priori expectations do not allow a prediction of the pattern of amino acid absorption since, as will be discussed below, parasites seem to vary more than hosts in this regard.
- (b) Free amino acids available to S. mansoni may vary as a function of host feeding. Data available suggest that the level of amino acids in portal blood varies as a function of the feeding of a vertebrate.

- (c) Free purines and pyrimidines in host blood may be accumulated by S. mansoni to satisfy nutritional requirements. Since this parasite cannot synthesize purines, this method of acquisition of essential basis would be efficient.
- (d) The tegument is the major organ for sugar absorption. Experiments with ligated and unligated schistosomes indicate that this is true for glucose. It seems probable that it is true for other sugars of physiological significance.
- (e) Sugar transport is the limiting step in carbohydrate metabolism in schistosomes. At concentrations approximately those of blood, schistosomes do not accumulate sugars.
- (f) Sugar transport may have tight constraints, in terms of the systems allowing movement of an energy source into the tissues of the parasite. There are a very limited number of mechanisms for sugar transport. The specificity may differ sharply from that of the kinases involved in sugar metabolism and differ from the sugar transport specificities of the host.

#### Background.

##### a. Basis

Our studies have indicated that the specificity of amino acid transport systems in S. mansoni differs sharply (1) from those of the host and (2) from those of other animal parasites which have been studied. Further, we have found it difficult to accept the idea, espoused by others, that this parasite would derive its amino acids for protein synthesis from digestion of blood proteins when free amino acids are available in the environment. If it obtains nutritional benefit from the macromolecules of blood, it further stretches our imagination to assume that hemoglobin is a favored protein source: Other proteins of blood are more nearly complete with respect to amino acid composition. In short, the prevalent views on the amino acid and protein nutritional of schistosomes are quite inadequate. There is a large literature relating to the nutritional problems of insects feeding on the peripheral blood of mammals. Various arthropods harbor symbiotes who furnish compounds which are not available in sufficient quantity in the peripheral blood of vertebrates. On the other hand, schistosomes, living in the portal blood, may have escaped these constraints.

#### Methods.

We have worked out a number of new "methods" in this research project. For example, the incubation of schistosomes for short intervals of time to determine uptake of labeled amino acids or sugars can be carried out in baskets consisting of a short piece of large glass tubing to one end of which we fasten a stainless steel or nylon mesh. This can be put into or removed from an incubation medium. The worms can be rapidly washed in such a device. Further, we have improved the "Perf-O-Suction" technique for recovery of schistosomes from the host, using mechanical components costing less than \$5.00. (Pappas and Asch, 1972)



The specific methods to be used in our experiments initially involve short term incubations of worms with isotopically labeled amino acids in the presence and absence of other amino acids. A detailed study of interactions of amino acids has been carried out, using ligation techniques to determine whether absorption occurs through the tegument and/or the gut of the worm. We have defined, in a precise fashion, the qualitative character of systems involved in the transport of amino acids into the tissues of S. mansoni by examining the interactions of amino acids in transport. Simultaneously, studies have determined free amino acids available to the parasite in the portal blood of the host.

Similar studies have been carried out with sugars to obtain a more precise evaluation of the specificity of sugar transport systems and the relation of transport to metabolism.

#### Results.

Six significant publications have appeared in relevant scientific journals under the auspices of this contract. All of these documents have added to our knowledge of schistosome nutrient acquisition.

Reprints of these publications have been forwarded to the relevant offices.

1. Asch, H. L. and C. P. Read. 1975. Transtegumental Absorption of Amino Acids by male Schistosoma mansoni. J. Parasitol. 61:378-379.

Proline and glycine enter S. mansoni through the tegument. This mode of nutrient acquisition is the predominant means of transport for these and possibly other low molecular weight compounds.

2. Asch, H. L. and C. P. Read. 1975. Membrane Transport in Schistosoma mansoni: Transport of amino acids by adult males. Exptl. Parasitol. 38:123-135.

The transport constants ( $K_t$ ) and maximal velocities ( $V_{max}$ ) for six amino acids (glycine, proline, methionine, arginine, glutamate and tryptophane) which entered S. mansoni in part by a mediated system were determined. Cysteine permeation was by diffusion only while proline had no diffusion component. All other amino acids entered the parasite by both diffusion and mediated systems. In the two minute incubations used in these experiments no metabolism of the amino acids was observed.

3. Asch, H. L. 1976. Amino Acid Pools of Schistosoma mansoni and mouse hepatic portal serum. Rice University Studies 62:35-42.

Twenty five ninhydrin positive compounds were identified in ethanolic extracts of the parasites. Ten to 13 unidentified compounds were also observed in these extracts. The levels of amino acids in the hosts serum approximated the respective transport constants ( $K_t$ ) for the parasite tegumental transport systems. Analysis of amino acid concentrations in the parasite and its environment suggests that some amino acids (especially glutamate) may be concentrated

against a potential difference.

4. Levy, M. G. and C. P. Read. 1975. Purine and Pyrimidine Transport in Schistosoma mansoni. J. Parasitol. 61:627-631.

Cytosine, thymine and uracil enter the parasites entirely by diffusion. Adenine, guanine, hypoxanthine and the nucleosides adenosine and uridine were accumulated in part by mediated transport mechanisms. The results suggest the presence of five distinct loci for transport: 1) guanine-hypoxanthine, 2) adenine-adenosine, 3) adenine, 4) adenosine-uridine and 5) adenosine. Inhibition or derangement of the transport of these essential compounds may be of clinical importance.

5. Levy, M. G. and C. P. Read. 1975. Relation of Tegumentary Phosphohydrolase to purine and pyrimidine Transport in Schistosoma mansoni. J. Parasitol. 61:648-656.

Inhibition of the transport of adenine or adenosine by AMP in adult S. mansoni suggested hydrolysis of the nucleotide at the tegumental surface and interaction of the liberated adenosine with the accumulation of adenine or adenosine. The inhibition resulting from hydrolysis was prevented by p-nitrophenol phosphate or ammonium molybdate--both inhibitors of the phosphohydrolase. Glucose-1-phosphate, glucose-6-phosphate, NaF or cysteine did not relieve the inhibition. AMP, ATP, UMP AND p-nitrophenyl phosphate were hydrolyzed by the tegument and surface enzyme(s).

6. Uglem, Gary L. and C. P. Read. 1975. Sugar Transport and metabolism in Schistosoma mansoni. J. Parasitol. 61:390-397.

Fructose and 3-O methyl glucose enter S. mansoni only by diffusion, while glucose, 2-deoxyglucose, galactose, glucosamine and mannose entered by both mediated and diffusion systems. Absorbed glucose was rapidly metabolized whereas 2-deoxyglucose was slowly metabolized and accumulated against a potential difference. The transport of 2-deoxyglucose was sensitive to decreased  $\text{Na}^+$ , ouabain, phlorizin, phloretin and other sugars. Studies with  $\text{Na}^{22}$  suggest that the accumulation of 2-deoxyglucose and  $\text{Na}^+$  were coupled.

#### Conclusions.

(a) Significant amounts of free amino acids permeate adult S. mansoni by mediated and diffusion modes.

(b) Mediated processes are specific and exhibit competition with closely related molecular species.

(c) The major sites of accumulation are the tegumental surface in lieu of the cecal epithelium.

(d) The absolute concentration of amino acids in the hepatic portal circulation of the host increase following feeding; however, the relative ratios of amino acids change very little.

(e) Strategies of nutrient acquisition of parasite do not significantly differ from intestinal mechanism of the host.

(f) Like amino acids the sugars enter adult S. mansoni via the tegument by both diffusion and mediated transport.

(g) Glucose is metabolized rapidly (<2 min.) which suggests that the absorption mechanism may be the rate limiting step.

(h) No significant difference exists in sugar permeation between adult S. mansoni and the mouse host.

(i) Purine and pyrimidine enter the tegument adult parasites.

(j) Phosphohydrolase(s) are responsible for nucleotide hydrolyses at the tegumental surface of the parasites.

(k) That adult Schistosoma cannot synthesize purines may afford an avenue for antiparasitic agents.

#### Recommendations.

(1) The similarity of permeation of both sugars and amino acids in adult Schistosoma mansoni and its mouse host suggests that interruption or rearrangement of this physiological mechanism is not the method of choice in developing a new antihelminthic.

(2) The requirement of purine in the biology of Schistosoma and the novel strategies of accumulation utilized by this parasite for those organic compounds indicates that further study on the requirements and metabolism of these bases may be a fruitful area of research toward antihelminthic development.

#### Literature Cited

- Isseroff, H. and C. P. Read. 1969. Studies on membrane transport. VI. Absorption of amino acids by fascioliid trematodes. *Comp. Biochem. Physiol.* 30: 1153-1159.
- Pappas, P. and H. Asch. 1972. Modification of the Perf-O-Suction technique for schistosome recovery. *Int. J. Parasitol.* 2:283.

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